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Silver nanoparticles: Antibacterial activity against wound isolates & *invitro* cytotoxic activity on Human Caucasian colon adenocarcinoma

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ABSTRACT

Objective: To synthesize the silver nanoparticles (AgNPs) using the extracts of *Hypnea sp*. and to investigate the antibacterial activity against *Eshcherichia coli*, *Staphylococcus aureus* and *invitro* cytotoxic activity on HT-29. **Methods:** In the present study, AgNPs were synthesized using the aqueous extract of marine macro-algae, and were characterized using UV-visible spectroscopy, Fourrier Transform Infra red (FT-IR) spectroscopy, X-ray diffraction (XRD) analysis and Transmission Electron Microscopy (TEM) analysis. Further these synthesized AgNPs were evaluated for their antibacterial activity with the clinical isolates from wound specimens. The isolates were characterized by different tests viz., microscopical observation, colony morphology, biochemical & sugar fermentation tests. The synthesized AgNPs were tested for its antibacterial activity against the isolates by agar well diffusion method. The AgNPs were assessesd for its cytotoxic activity on Human Caucasian colon adenocarcinoma (HT-29) cell lines. **Results:** In this study, it is clear that the synthesized AgNPs were spherical measuring 10-20nm and was found to be more bactericidal against Gram-negative bacteria (*E. coli*) than Gram-positive bacteria (*Staphylococcus aureus*) isolated from wound specimen. The *invitro* screening of the AgNPs showed potential cytotoxic activity against the colon cancer cell lines. **Conclusions:** Proteins can bind to nanoparticles either through the electrostatic attraction of negatively charged carboxylate groups in *Hypnea sp*. and stabilization of the AgNPs by protein occurs. The antimicrobial activities of AgNPs are influenced by the dimensions of the particles the smaller the particles, the greater antimicrobial effect. The cytotoxic activity may be due to the presence of alkaloids present in the *Hypnea sp*.

1. Introduction

Nanoparticles with controlled size are of technological interest as they provide technological challenges in various fields particularly in medical field. Nanomaterials due to their small size show unique and considerably changed physical, chemical, and biological properties compared to their macro scale counterparts [1]. AgNPs have been produced using different methods: electrochemical method, laser ablation, microwave irradiation, thermal decomposition and sono-chemical synthesis [2, 3]. Although chemical and physical methods may successfully produce pure, well-defined nanoparticles, these methods are quite expensive and potentially dangerous to the environment. Considering that such reducing agents may be associated with environmental toxicity or biological hazards. The development of a green synthesis for AgNPs is desired. Use of biological organisms such as microorganisms, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner [4]. Thus a green macro-alga, *Hypnea sp*. was used to synthesize the AgNPs in an eco-safe method.

Nowadays multiple drugs resistance has developed due to the use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. Antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. Therefore there is a need to develop new antimicrobial drugs for the treatment of infectious diseases. Antimicrobials of marine plant origin have enormous therapeutic potential [5]. The beneficial medicinal effects

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result from the combinations of secondary products present in the marine plant [6]. Because of their high reactivity due to the large surface to volume ratio, nanoparticles play a crucial role in inhibiting bacterial growth in aqueous and solid media. The antimicrobial activities of AgNPs are influenced by the dimensions of the particles the smaller the particles, the greater antimicrobial effect [7]. It is generally recognized that AgNPs may attach to the cell wall, thus disturbing cell-wall permeability and cellular respiration. The nanoparticles may also penetrate inside the cell causing damage by interacting with phosphorus and sulfur containing compounds such as DNA and protein. Another possible contribution to the bactericidal properties of AgNPs is the release of silver ions from particles [8].

In this study the antibacterial activity of Ag nanoparticles synthesized using the green algae-*Hypnea sp*. against aerobic wound isolates (Gram negative bacteria-*Escherichia coli* and Gram positive bacteria-*Staphylococcus aureus*) was assessed by well diffusion method. Infections in deep wounds and abscesses are often caused by a mixture of aerobic and anaerobic organisms [9]. Wound swabbing involves the use of a cotton-tipped swab to sample superficial wound fluid and tissue debris and enables a semiquantitative and qualitative analysis of the wound microflora [10]. Although the value of acquiring superficial swab samples has been questioned, the procedure is simple, inexpensive, non-invasive and convenient for the majority of wounds [11]. The aim of this study was to determine the most common bacteria isolated from wound specimen and to evaluate the antibacterial activity of the synthesized AgNPs.

The fight against cancer has not been successful so far, particularly in the development of therapies for rapidly growing tumors. It is a challenge to find drugs for the effective treatment of various types of cancer. Cancer causes significant morbidity and mortality and is a major health problem worldwide. HT29 cells are human intestinal epithelial cells which produce the secretory component of Immunoglobulin A (IgA), and carcinoembryonic antigen (CEA). Cells are used for tumourigenicity studies. The incidence of colon cancer is rising in every country of the World. It is the fourth most common cause of cancer death after lung cancer, stomach cancer and liver cancer. Thus, colon cancer is a worldwide disease and needs to be addressed seriously. Medicines derived from plants have played a pivotal role in health care of ancient and modern cultures. In recent years, an increasing number of marine natural products have been reported to display antimicrobial activities and anti-tumor compounds have been isolated from sponges, tunicates, algae and marine plants [12]. In most cases, the evaluation of the anti-cancer potential of crude extracts from different sea organisms has been carried out by *in vitro* cytotoxicity tests in malignant cell cultures [13]. Hundreds of potential anti-tumor agents have been isolated from marine origin especially from marine algae [14]. Isolation of cytotoxic anti-tumor substances from marine organisms has been reported in several references during the last 40 years, while in recent years, hundreds of bio-potential anti-tumor agents have been isolated from marine origin especially from marine algae [15].

In this study, AgNPs were synthesized using *Hypnea sp*. and their antibacterial activity and *invitro* cytotoxic activity was assessed.

2.Materials and method

2.1.Synthesis of silver nanoparticles

1 g of *Hypnea sp*. powder was extracted aqueously and filtered with Whatmann no.1 filter paper. The extract was stored at 40 ℃ until use. 1mM silver nitrate solution was added to the filtrate under magnetic stirring conditions and subjected to 121℃ for 10 mins. The extract is used as reducing and stabilizing agent for 1mM of Silver nitrate. The appearance of a brownish colour in solution is a clear indication of the formation of AgNPs in the reaction mixture [16]. Simultaneously control without silver ions was also run along with the experimental flask [17].

2.2.Characterization of AgNPs

The reduction of pure Ag_+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium using UV-1601 pc shimadzu spectrophotometer. The interaction between protein-silver nanoparticles was analyzed by FTIR. FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of the Ag_+ ions and the capping of the bioreduced AgNPs synthesized by seaweed extract. For FT-IR measurements, both the seaweed extract and the reduced solution were analyzed on a Perklin-Elmer FT-IR instrument in the diffuse reflectance mode at a resolution of $4cm^{-1}$. TEM analysis was employed to visualize the size and shape of AgNPs. TEM measurements were performed using TENCAI 10, Philips instrument. X-ray diffraction (XRD) measurement of the seaweed reduced AgNPs was carried out using powder X-ray diffractometer instrument (PXRD-6000 SCHIMADZU) in the angle range of 10℃-80℃ at 2θ, scan axis: 2:1 sym. The size of the AgNPs was calculated from the PXRD peak positions using Bragg's law.

2.3.Isolation and characterization of organisms from wound specimen

Gram negative bacteria-*Escherichia coli* and Gram positive bacteria-*Staphylococcus aureus* were isolated from wound specimen and characterized using appropriate tests. The isolates were confirmed by morphological (staining and motility), cultural (Mac Conkey agar, Eosin Methylene Blue, Blood agar, Mannitol salt agar), biochemical tests (IMIC test,

triple sugar iron test, catalase test, oxidase test, and sugar fermentation tests) and Coagulase test [9].

2.4.Antibacterial activity of AgNPs

The bacterial isolates were grown in nutrient broth for 24 h. A 100 ml nutrient broth culture of each isolate (1×10^5cfu/ml) was used to prepare bacterial lawns. Antibacterial activity of the synthesized AgNPs was determined using the agar well diffusion assay method [18]. Approximately 20 ml of molten and cooled Mueller Hinton agar media was poured in sterilized petri dishes. The plates were left overnight at room temperature to check for sterility. The isolates were swabbed onto the plates. Agar wells of 5 mm diameter were prepared with the help of a sterilized cork borer. As a preliminary qualitative assay, two wells were bored, one well containing the extract alone and the other well loaded with the synthesized AgNPs. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells.

As a quantitative method, four wells were prepared in Mueller Hinton agar plate. Different concentrations (5 μg/ml, 10 μg/ml, and 15 μg/ml) of the AgNPs were prepared in DMSO and added to the respective wells and a control was maintained with the fourth well. Then the plates were incubated at 37℃ for 24 hrs, where upon inhibitory activity was observed as a zone of clearing around the wells. The diameter of the clearing zones was measured in mm using the ruler scale. The experiment was done in triplicate for each pathogenic bacterium and compared with the standard antibiotic sensitivity chart.

2.5. Cell culture

HT29 cancer cell line was cultured in Dulbecco's Modified Eagle Medium (DMEM). All culture media were supplemented with 10% fetal bovine serum (FBS), 1% antibiotic and antimycotic solution (50,000 units/L of penicillin and 50 mg/L of streptomycin) and 2mM glutamine. Cultures were held in 75 cm culture flasks at 37 ℃, 5% CO and 95% relative humidity, changing media at least twice a week [19].

2.6.Evaluation of cytotoxic activity of the silver nanoparticles on HT-29 cell lines

Human colon cancer cell line (HT29) was seeded in 96-well tissue culture plates. Stock solutions of nanoparticles (5 mg/ml) were prepared in sterile distilled water and diluted to the required concentrations (10, 5, 2.5, 1.25, 0. 625, 0.312, 0.156 mg/ml) using the cell culture medium. Appropriate concentrations of Ag-NP stock solution were added to the cultures to obtain respective concentration of Ag-NP and incubated for 48 hrs at 37℃. Non-treated cells were used as control. Incubated cultured cell was then subjected to MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a tetrazole) colorimetric assay [20]. The tetrazolium salt 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) is used to determine cell viability in assays of cell proliferation and cytotoxicity. MTT is reduced in metabolically active cells to yield an insoluble purple formazon product. Cells were harvested from maintenance cultures in the exponential phase and counted by a hemocytometer using trypan blue solution. The cell suspensions were dispensed (100 μl) in triplicate into 96-well culture plates at optimized concentrations of 1.5 x 10 cells/ml for HT29, after a 24-hr recovery period. Assay plates were read using a spectrophotometer at 520 nm. Data generated were used to plot a dose-response curve of which the concentration of extract required to kill 50% of cell population (IC_{50}) was determined.

Cell viability (%) = Mean OD/ control OD × 100

Following Ag-NP treatment, the plates were observed under an inverted microscope to detect morphological changes and photographed.

3.Results

Reduction of silver ion into AgNPs during exposure to the aqueous extract of *Hypnea sp*. could be followed by color change (Fig.1.). The filtrate of the seaweed extract with 1mM silver nitrate solution when allowed to react at 121℃ for 10 min. changed to dark brown color solution. Control (without AgNO3) showed no color formation. This confirmed the formation of AgNPs. Fig.2. UV-Vis spectra shows no evidence of absorption in the range of 400-800 nm for the seaweed extract and the seaweed extract exposed to $AgNO_3$ ions shows a distinct absorption at around 429 nm.

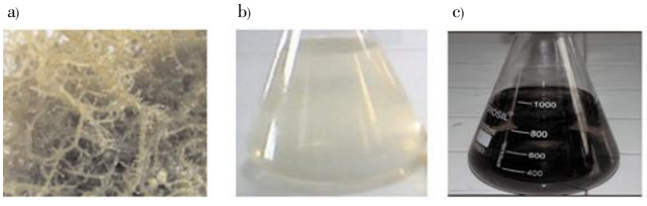

Fig. 1. Photography of a) *Hypnea sp*. b) Control c) Treated

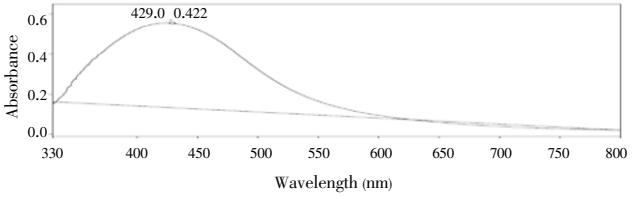

Fig. 2. UV-Vis spectrum of the aqueous extract of *Hypnea sp*. Vs. AgNPs.

FT-IR spectrum of the *Hypnea sp*. extract (Fig. 3) shows peaks at 3452cm-1, 2374cm-1, 2094cm-1,1631cm^{-1}, 1381cm^{-1} and 736cm-1, where as intense FT-IR bands of AgNPs were

inferred at 3448cm−1, 2077cm−1, 1638cm−1 and 713 cm^{-1}. The TEM technique used to visualize size and shape of the AgNPs as shown in Fig. 4. The XRD patterns of synthesized AgNPs shows four intense peaks in the whole spectrum of 2θ values ranging from 10° to 80°.

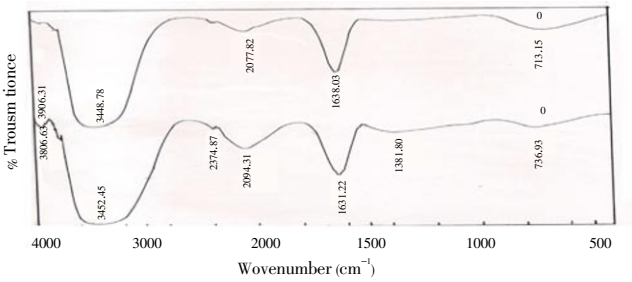

Fig. 3. FT-IR spectrum – a) extract of *Hypnea sp.* b) AgNPs

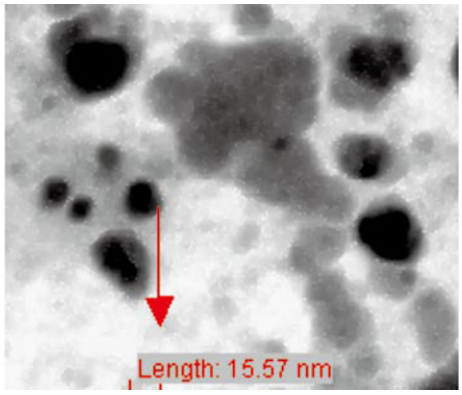

Fig. 4. TEM image of the AgNPs

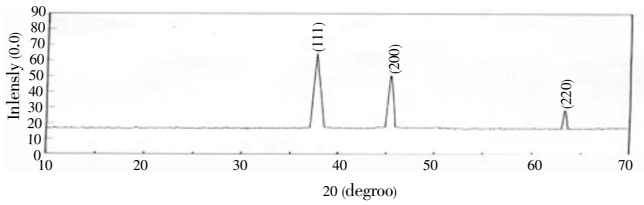

Fig. 5. XRD pattern of the synthesized AgNPs

The wound specimen was inoculated onto selective media, Eosin Methylene Blue (EMB) agar and on differential media-Mac Conkey Agar for isolation of Gram-negative *Escherichia coli* and on Mannitol Salt Agar (MSA) and Blood Agar for isolation of Gram-positive *Staphylococcus aureus*. On EMB agar, metallic sheen, and on Mac Conkey agar, pink color colonies were observed indicating the presence of *E. coli*. On MSA plate, yellow colonies and on Blood agar, β-hemolytic colonies were observed indicating the presence of *Staphylococcus aureus* (Fig. 6.). The isolates were identified and characterized by various biochemical tests and sugar fermentation tests and the experiment was done in triplicate for each isolate. On biochemical analysis, *E. coli* was positive for indole and methyl red test, negative for utilization of citrate and urease, in triple sugar iron medium glucose, sucrose and lactose were fermented acid were produced along with gas. Individual sugars like Glucose, lactose, maltose and sucrose were fermented along with acid and gas production.

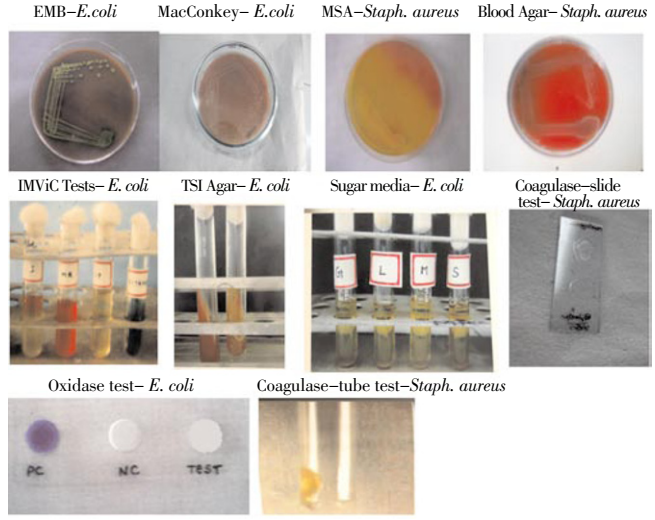

Fig. 6. Isolation and characterization of wound isolates-*Escherichia coli* and *Staphylococcus aureus*

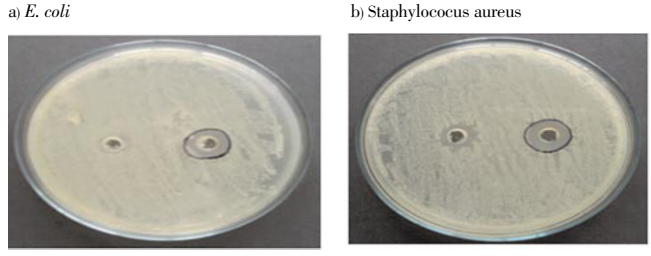

Fig. 7. Preliminary test for assessing the antimicrobial activity of the AgNPs

In the preliminary qualitative antibacterial assay, a good zone of inhibition of the wound isolates was observed. A control was maintained, where a small zone of inhibition was observed (Fig. 8.). In the quantitative assay, 5, 10, 15 μg/ml of AgNPs were added in three respective wells and a control was maintained in the fourth well. The zone of inhibition was measured as 20mm, 29mm, 32mm respectively in diameter. A Control was maintained using the standard drugs viz. Gentamycin, Chloramphenicol, Streptomycin and Ampicillin (Fig.9.).

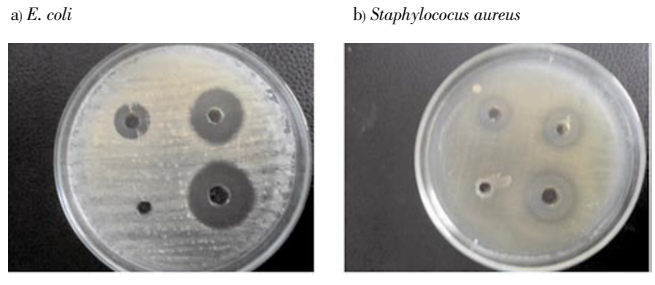

Fig. 8. Antibacterial activity of AgNPs using different concentrations (5 μg/ml, 10 μg/ml, 15 μg/ml)

a) *E. coli* b) *Staphylococus aureus*

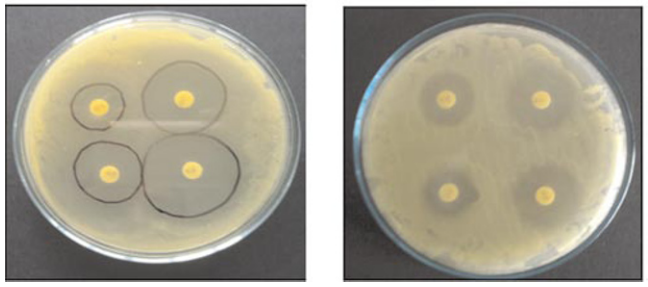

Fig. 9. Antibacterial activity against standard drugs (Gentamycin, Chloramphenicol, Streptomycin, Ampicillin)

Invitro cytotoxic activity against HT29 cell line at different concentrations was evaluated. The *invitro* screening of the AgNPs showed potential cytotoxic activity against the colon cancer cell lines. The results obtained are shown in Table 1 & Fig.10. At a concentration 1.25 mg/ml, 57.69% of cytotoxicity and at a concentration 2.5 mg/ml, 42.30% cytotoxicity was recorded.

Table 1

Cytotoxicity of AgNPs

S.NO	CONCENTRATION (mg/ml)	DILUTIONS	CELL VIABILITY
1	10	Neat	19.23±0.97
2	5	1:1	30.76±1.28
3	2.5	1:2	42.303±0.67
4	1.25	1:4	57.69±1.03
5	0.625	1:8	65.38±1.5
6	0.312	1:16	73.07±0.66
7	0.156	1:32	84.61±1.28
8	Cell control	–	100

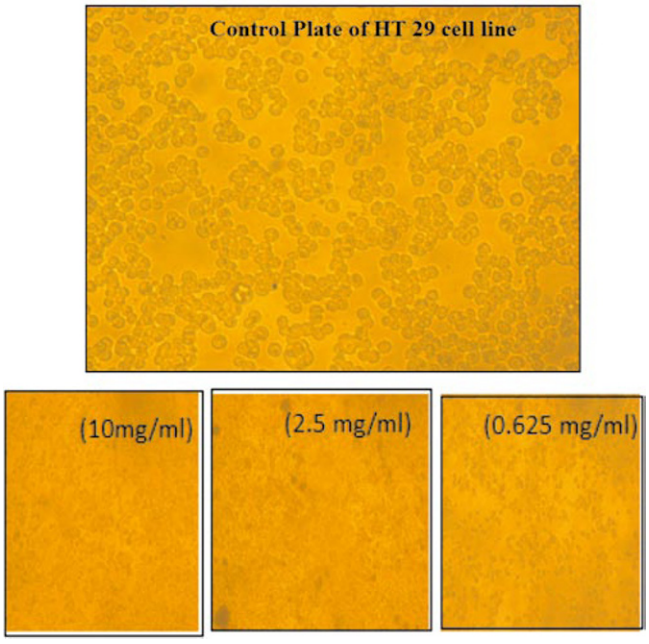

Fig. 10. Photography of the cytotoxicity on HT29 cell lines

4.Discussion

It is well known that silver nanoparticles exhibit a yellowish-brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles[21]. The formation of AgNPs is attributed to hydrophilic-hydrophobic interactions resulting in intermolecular forces [22]. UV-vis absorption spectra showed a strong peak at 429nm which corresponds to surface plasmon resonance of AgNPs established at 420 nm [23].

Curve of the seaweed extract resulted a strong band at 3452 cm^{-1} which corresponds to O-H stretching of carboxylic acids, 2374cm-1 corresponds to N-H stretching of secondary amines, 1631cm-1 corresponds to the O-H stretching of the primary amines, 1381cm-1 corresponds to the C-H stretching of the methyl group and a weak band at 713cm^{-1} corresponds to the C-N stretching of the fluoroalkanes. Curve of the AgNPs biosynthesized using the *Hypnea sp*. extract resulted in a strong band at 3448cm^{-1} corresponding to O-H stretching of carboxylic acids, 2077 cm-1 corresponds to C-N stretching, 1638cm-1 corresponds to C=N stretching of amines and 713cm-1 corresponds to C-H stretching of aromatic compounds. These findings support that proteins can bind to nanoparticles either through the electrostatic attraction of negatively charged carboxylate groups and therefore stabilization of the AgNPs by protein occurs [24].

The morphology of nanoparticles is spherical and the TEM micrograph suggests that particle diameters ranged from 10 to 20 nm. The dimensions of AgNPs were small enough to be electron transparent and imaged as poly dispersed small and large spherical nanoparticles with variable diameter [25]. Our investigation revealed that the edges of the particles were lighter than the centers, suggesting that biomolecules, such as proteins in *Hypnea sp*. capped the silver NPs.

The XRD pattern of 2θ=38.20°, 44.11°, and 64.35°are in agreement with the Joint Committee on Powder Diffraction Standards (file No. 04-0783), which further proves the formation of crystal AgNPs [26]. The peaks were identified as AgNPs according to PCPDFWIN software (PDF#030921) in Fig.5.

From this study, it is clear that the synthesized AgNPs were found to be more bactericidal against Gram-negative bacteria (*E. coli*). In contrast, AgNPs were found to have a less significant effect on the growth of gram-positive bacteria (S. aureus) [27, 28]. It has been reported the greater the zone of inhibition, greater the antibacterial properties of the AgNP and that antibacterial effect was size and dose dependant and was more pronounced against Gram-negative bacteria than Gram-positive bacteria [29, 30]. Reports on the inhibitory action of silver ions on microorganisms show that upon silver ion treatment, DNA loses its replication ability and expression of ribosomal subunit proteins as well as some other cellular proteins and enzymes essential to ATP

production becomes inactivated [31]. It was well known that AgNPs exhibit strong antibacterial activity due to their well developed surface which provides maximum contact with the environment. The inhibition of bacterial growth reported in this study is dependent on the concentration of AgNPs in the medium. The AgNPs synthesized using the marine microalgae was reported for its antibacterial agent by Devina Merin et al [17].

In this study, it was observed that the synthesized AgNPs induces a concentration dependent inhibition of HT29 cells. Almost 60% of drugs approved for cancer treatment are of natural origin [32]. Therefore there is an urgent need to develop alternative therapeutic measures against this deadly disease. The new age drugs like nanoparticles of polymers, metals or ceramics, which can combat conditions like cancer [33] and fight human pathogens like bacteria [34]were discovered.

5.Conclusion

The silver nanoparticles were synthesized using the aqueous extract of marine maroalgae- *Hypnea sp*. The synthesized AgNPs were characterized using UV vis spectroscopy, FT-IR, XRD, TEM analyses. The synthesized AgNPs were assessed for its antibacterial and *invitro* cytotoxic activity. The Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* were isolated and were characterized using appropriate tests. The AgNPs were highly efficient bactericidal agent against Escherichia coli. The cytotoxic activity on HT 29 colon cell lines may be due to the presence of alkaloids present in the *Hypnea sp*.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

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References

[1] Virender K. Sharma, Ria A. Yngard, Yekaterina Lin. Silver nanoparticles: Green synthesis and their antimicrobial activities. *Advances in Colloids and Interface Science* 2009; **145**: 83-96.

[2] Patel K, Kapoor S, Dave DP, Mukherjee T. Synthesis of nanosized silver colloids by microwave dielectric heating. *J. Chem. Sci.* 2007; **117** (4):311-314.

[3] Saifuddin N, Wong W, Nur Yasumira AA. Rapid Biosynthesis of Silver Nanoparticles Using Culture Supernatant of Bacteria with Microwave Irradiation. *E-Journal of chemistry* 2009; **6**(1):61-70.

[4] Prabakaran M, Subha K, Thennarasu V, Merinal S. Biosynthesis of silver nanoparticles using Sphaerulina albispiculata and evaluation of antibacterial activity. *European Journal of Experimental Biology* 2012; **2** (1):297-303.

[5] Jain D, Daima, HK, Kuchhwaha S, Kothari SL. Plant mediated silver Nanoparticles using papaya fruit extract and evalution of their antimicrobial activites. *Digest journal of nanomaterials and biostructures* 2009; **4**: 557-563.

[6] Valentin Bhimba B, Meenupriya J, Elsa Lycias Joel, Edaya Naveena D, Suman Kumar, Thangaraj T. Antibacterial activity and characterization of secondary metabolites isolated from mangrove plant *Avicennia officinalis*. *Asian Pacific Journal of Tropical Med* 2010; **3**(7):544- 546.

[7] Shahverdi AR, Fakhimi A, Shahverdi HR, Minian MS. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. *Nanomedicine* 2007; **3**: 168-171.

[8] Sambhy V, MacBride MM, Peterson BR, Sen A. Silver bromide nanoparticle/polymer composites: Dual action tunable antimicrobial materials. *J Am Chem Soc* 2006; **128**: 9798-9808.

[9] Patrick R, Murray, Ellen Jo Baron, James H. Jorgensen, Marie Louise Landry, Michael A. Pfaller. Manual of Clinical Microbiology, 9th ed., *American Society of Microbiology Washington*, DC (2007).

[10]Kirketerp Moller K. Distribution, organization, and ecology of bacteria in chronic wounds. *J Clin Microbiol*. 2008; **46** (8):2717-2722.

[11]Kingsley A. A proactive approach to wound infection. *Nurs Stand*. 2001; **15**(30): 50-58.

[12]Elsa Lycias Joel, Valentin Bhimba B. Isolation and characterization of secondary metabolites from the mangrove plant *Rhizophora mucronata*. *Asian Pacific Journal of Tropical Med*. 2010; **3** (8):602-604.

[13]Valentin Bhimba B, Agnel Defora Franco DA, Jibi Merin Mathew, Geena Mary Jose, Elsa Lycias Joel, Thangaraj M. Anticancer and antimicrobial activity of mangrove derived fungi *Hypocrea lixii* VB1. *Chineese journal of natural medicine* 2012; **10**(1):77-80.

[14]Valentin Bhimba B, Vinod V, Cindhu Beulah M. Biopotential of secondary metabolites isolated from marine sponge *Dendrilla nigra*. *Asian Pacific Journal of Tropical Disease* 2011; **1**(4):299- 303.

[15]Chakraborty K, Lipton AP, Raj RP, Vijayan KK. Antibacterial labdane diterpenoids of *Ulva fasciata* Delile from southwestern

coast of the Indian Peninsula. *Food Chem* 2010; **119**: 1399-1408.

[16]Afreen Banu, Vandhana Rathod, Ranganath E. Silver nanoparticle production by Rhizopus stolonifer and its antibacterial activity against extended spectrum β-lactamase producing (ESBL) strains of Enterobacteriaceae. *Materials Research Bulletin* 2011; **46** (9):1417-1423.

[17]Devina Merin, Prakash S, Valentin Bhimba B. Antibacterial screening of silver nanoparticles synthesized by marine microalgae. *Asian Pacific Journal of Tropical Medicine* 2010; **3** (9):797-798.

[18]Khan S.S., Mukherjee A., Chandrasekaran N. Studies on interaction of colloidal silver nanoparticles (SNPs) with five different bacterial species. *Colloid Surface B.* 2011; **87**:129-138.

[19]Bishnu JL, Sunil, Anuja S. Antibacterial Property of Different Medicinal Plants: *Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum*, *Kathmandu University J. Science, Engineering and Technol.*, 2009; **5**:143-150.

[20]Ian Freshney. Culture of Animal Cells: a Manual of Basic Techniques. Fourth Edition. *New York, John Wiley and Sons,* 1994; 330-331.

[21]Guangquan Li, Dan He, Yongqing Qian, Buyuan Guan, Song Gao, Yan Cui, Koji Yokoyama, and Li Wang. Fungus-Mediated Green Synthesis of Silver Nanoparticles Using *Aspergillus terreus* Int *J Mol Sci* 2012; **13**(1):466-476.

[22]Medina-Ramirez T, Bashir S, Luo Z, Liu JL. Green synthesis and characterization of polymer-stabilized silver nanoparticles. Colloids Surf. B. *Biointerfaces* 2009; **73**:185-191.

[23]Kora AJ, Sashidhar RB, Arunachalam. Gum kondagogu (Cochlospermum gossypium): A template for the green synthesis and stabilization of silver nanoparticles with antibacterial application. *J Carbohydr Polym*. 2010; **82**: 670-679.

[24]Gole A, Dash C, Ramakrishnan R, Sainkar SR, Mandale AB, Rao M. Pepsin-gold colloid conjugates: preparation, characterization, and enzymatic. *Langmuir* 2001; **17**: 1674-9.

[25]Ahmad A, Mukherjee P, Senapati S, Mandal D, Islam Khan M, Kumar R. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids and *Surfaces B*: *Biointerfaces* 2003; **28 (4)**: 313-318.

[26]Paneerselvam C, Ponarulselvam S, Murugan K, Kalimuthu K, Thangamani S. Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* Linn. G.Donn and their antiplasmodial activities. *Asian Pacific Journal of Tropical Biomedicine* 2012; 1-7.

[27]Sarmistha Ray, Swadesh Sarkar, Surekha Kundu. Extracellular Biosynthesis Of Silver Nanoparticles Using The Mycorrhizal Mushroom Tricholoma Crassum (Berk.) Sacc.: Its Antimicrobial Activity Against Pathogenic Bacteria And Fungus, Including Multidrug Resistant Plant And Human Bacteria. *Digest Journal of Nanomaterials and Biostructures* 2011; **6** (3):1289-1299

[28]Geethalakshmi R, Sarada DVL. Synthesis of plant mediated silver nanoparticles using *Trianthema decandra* extract and evaluation of their antimicrobial activities. *Int J Eng Si Tech* 2010; **2** (5) 979-975.

[29]Khan SS, Mukherjee A, Chandrasekaran N. Studies on interaction of colloidal silver nanoparticles (SNPs) with five different bacterial species. *Colloid Surface B*. 2011; **87**:129-138.

[30]Fayaz, AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. *Nanomedicine* 2010; **6**:103-109.

[31]Hemath Naveen K.S., Gaurav Kumar, Karthik L., Bhaskara Rao K.V. Extracellular biosynthesis of silver nanoparticles using the filamentous fungus *Penicillium* sp. *Archives of Applied Science Research* 2010; **2** (6):161-167.

[32]B.Valentin Bhimba, D.A. Agnel Defora Franco, Geena Mary Jose, Jibi Merin Mathew, Elsa Lycias Joel. Characterization of Cytotoxic compound from Mangrove derived fungi *Irpex hydnoides* VB4. *Asian Pacific Journal of Tropical Biomedicine* 2011; **1**(4):223-226.

[33]Gutierrez FMPL, Olive A, Banuelos E, Orrantia N, Nino E, Sanchez M, Ruiz F, Bach H, Gay YA. Synthesis, characterization, and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. *Nanomedicine* 2010; **6**: 681-688.

[34]Ratan Das, Sneha Gang, Siddhartha Sankar Nath. Preparation and antibacterial activity of silver nanoparticles. *Journal of Biomaterials and nanobiotechnology* 2011; **2**: 472-475.